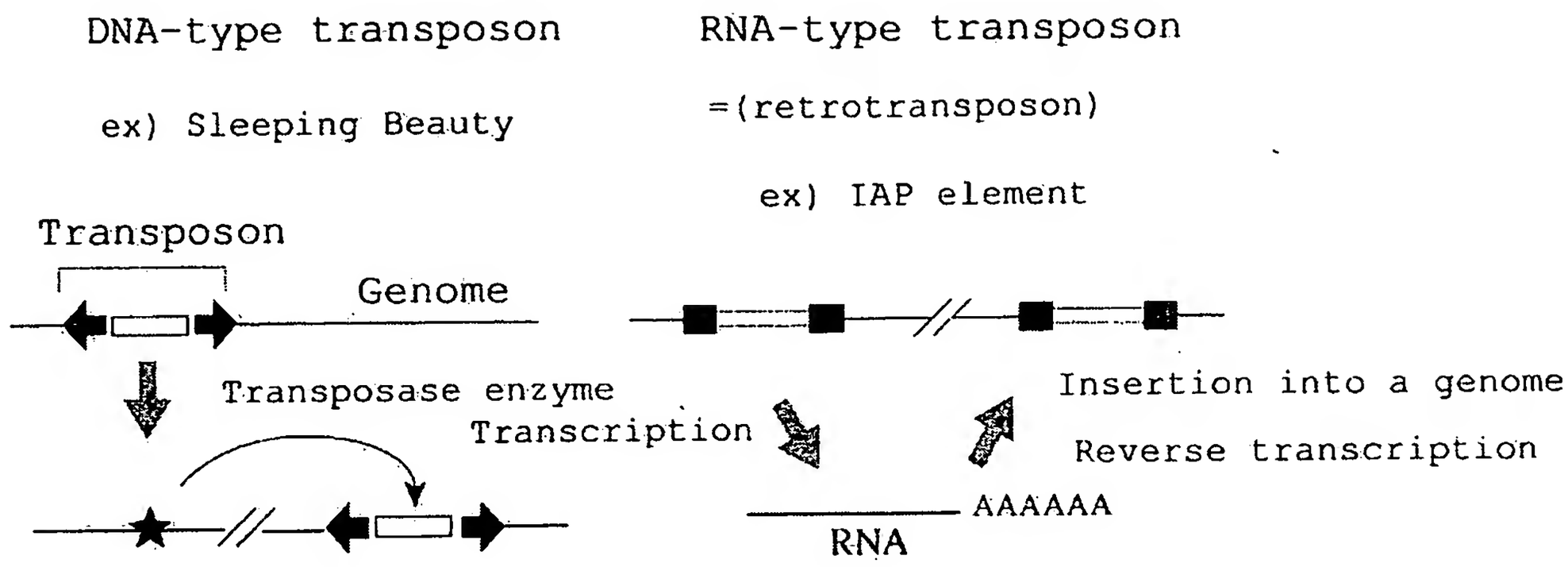

DNA-type transposon
ex) Sleeping Beauty
RNA-type transposon
=(retrotransposon)
ex) IAP element
Transposon
Genome
Transposase enzyme
Transcription
Insertion into a genome
Reverse transcription
AAAAAA
RNA

Fig. 1

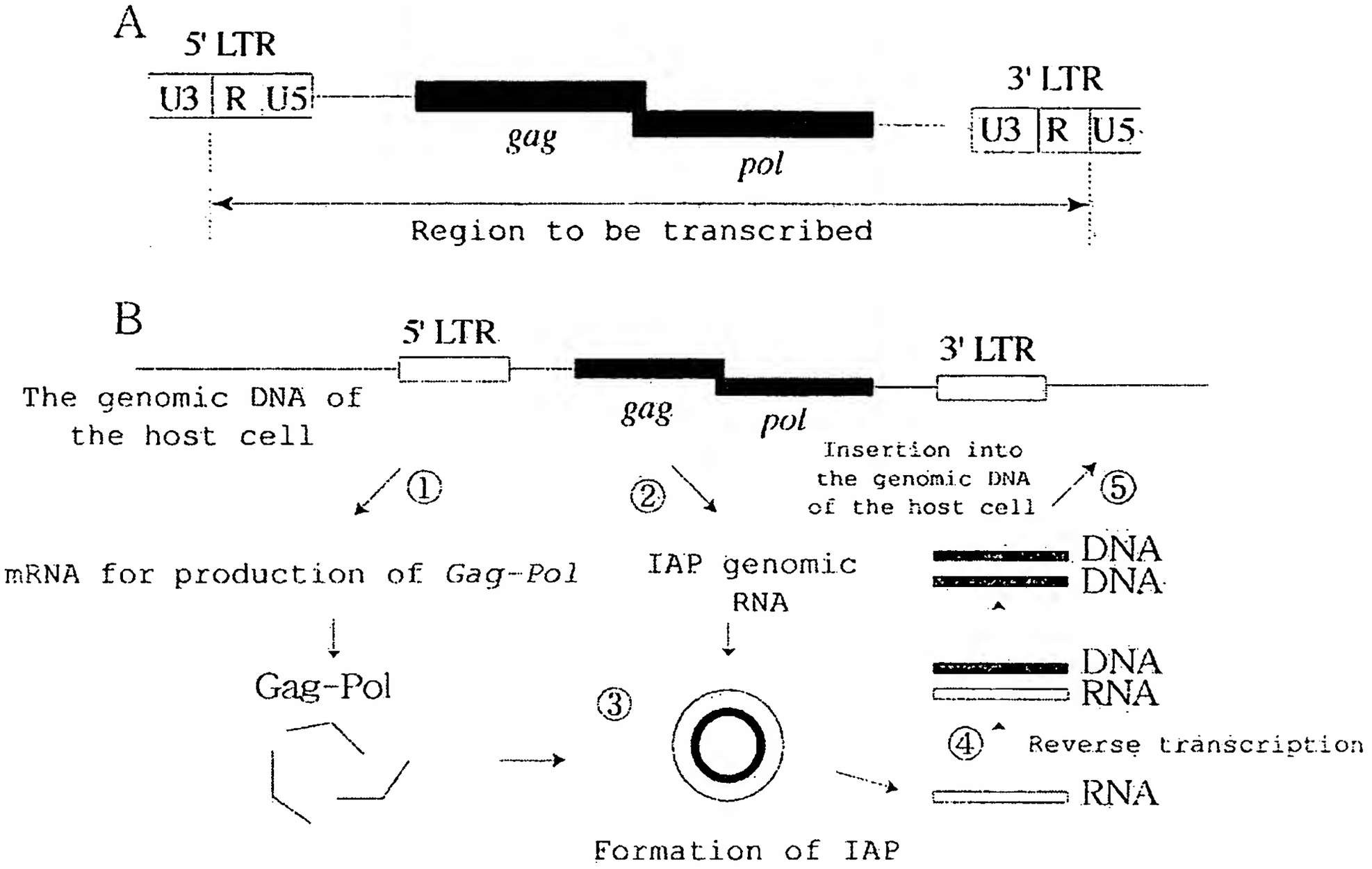
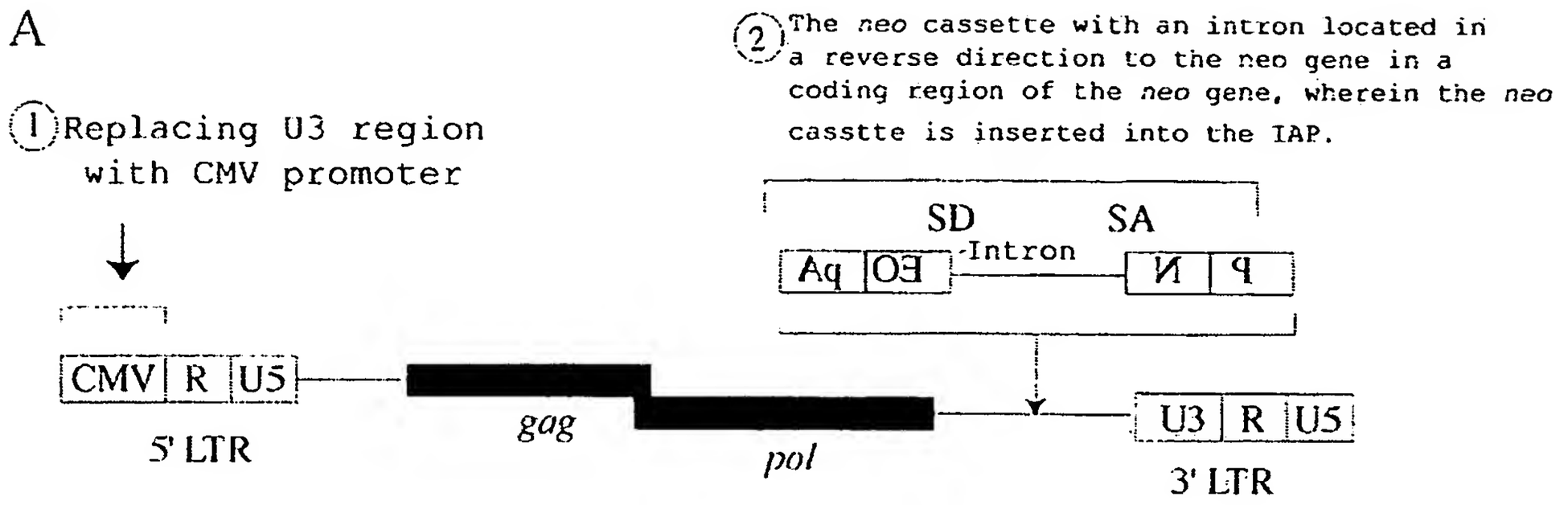
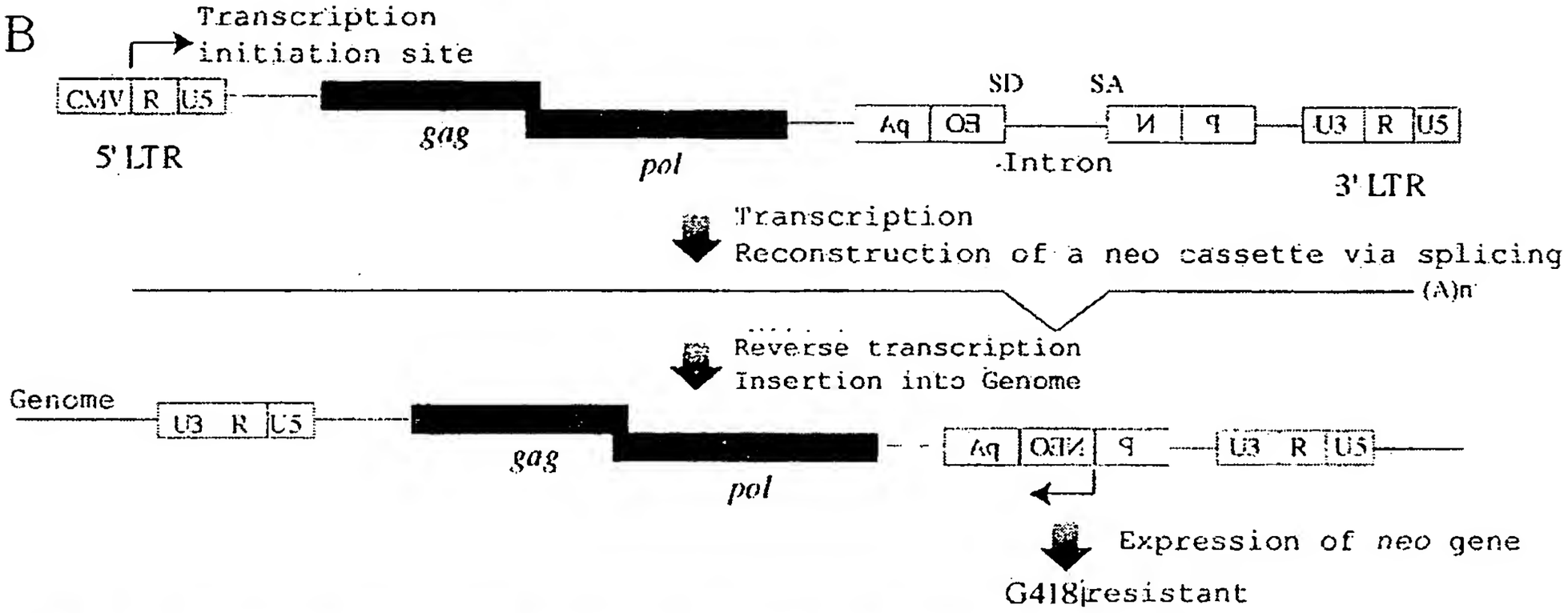

A
5' LTR
U3
R
U5
gag
pol
3' LTR
U3
R
U5
Region to be transcribed
B
5' LTR
3' LTR
The genomic DNA of the host cell
gag
pol
①
②
Insertion into the genomic DNA of the host cell
⑤
mRNA for production of Gag-Pol
IAP genomic RNA
DNA
DNA
Gag-Pol
③
DNA
RNA
④ Reverse transcription
RNA
Formation of IAP

Fig. 2


A
① Replacing U3 region with CMV promoter
② The neo cassette with an intron located in a reverse direction to the neo gene in a coding region of the neo gene, wherein the neo casstte is inserted into the IAP.
SD
SA
Intron
CMV
R
U5
5' LTR
gag
pol
U3
R
U5
3' LTR


B
Transcription initiation site
CMV
R
U5
5' LTR
gag
pol
SD
SA
Intron
U3
R
U5
3' LTR
Transcription
Reconstruction of a neo cassette via splicing
(A)n
Reverse transcription
Insertion into Genome
Genome
Expression of neo gene
G418 resistant

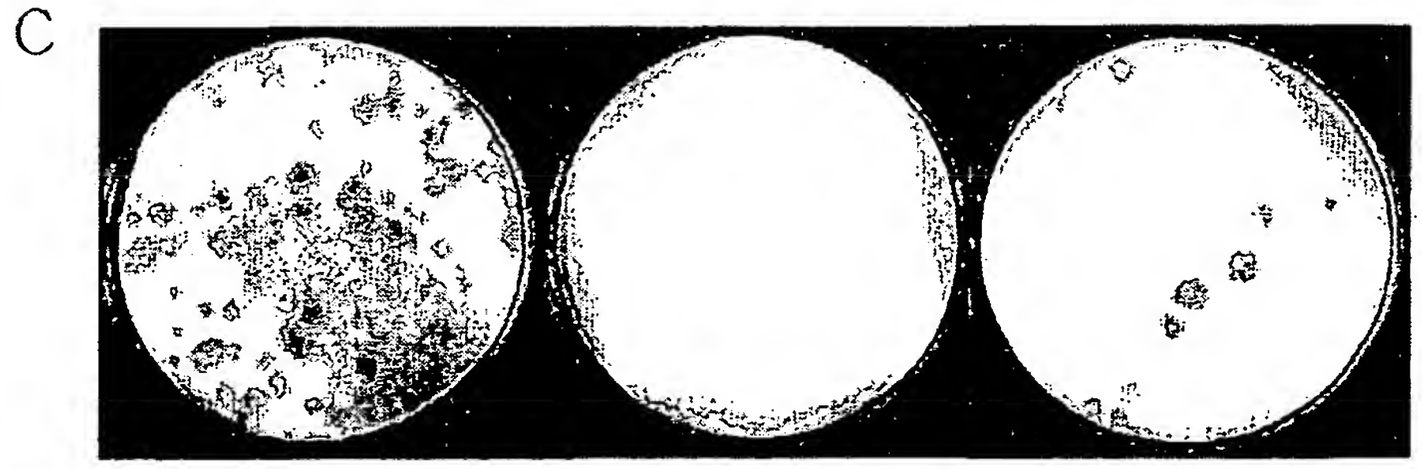
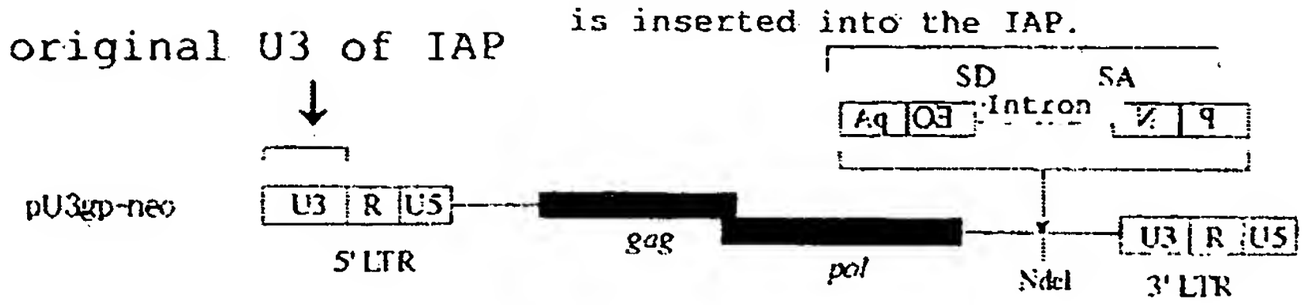

C
(i) IAP
(ii) IAP-delta pol
(iii) LINE1

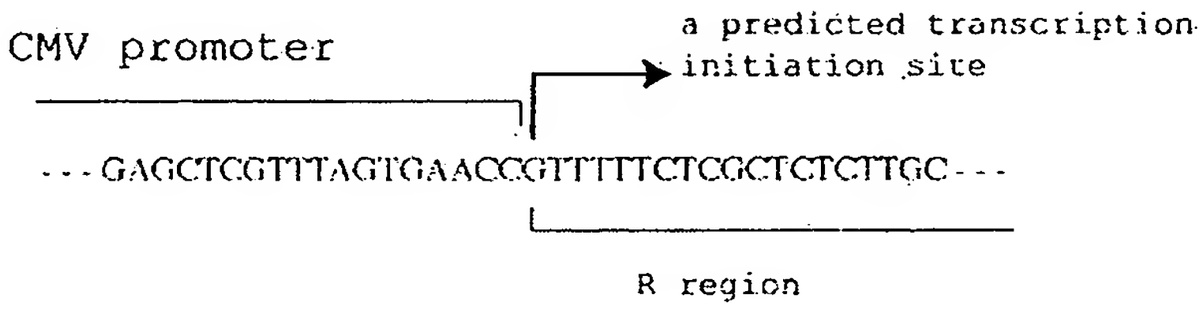
Fig. 3

A

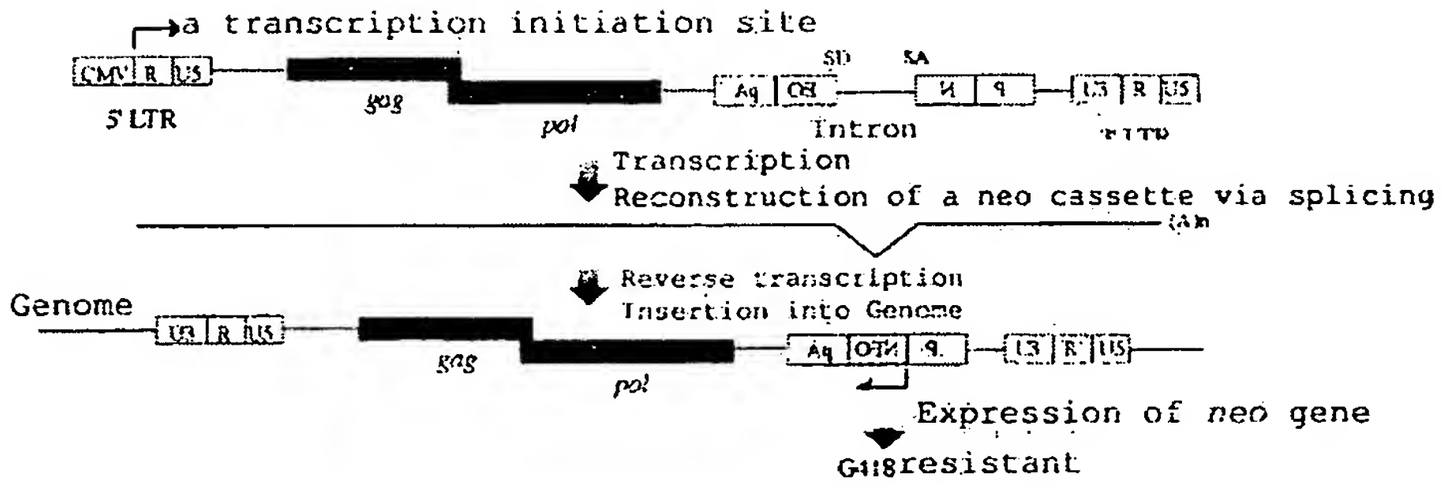
The *neo* cassette with an intron located in a reverse direction to the neo gene in a coding region of the *neo* gene, wherein the *neo* casstte is inserted into the IAP.



B



C

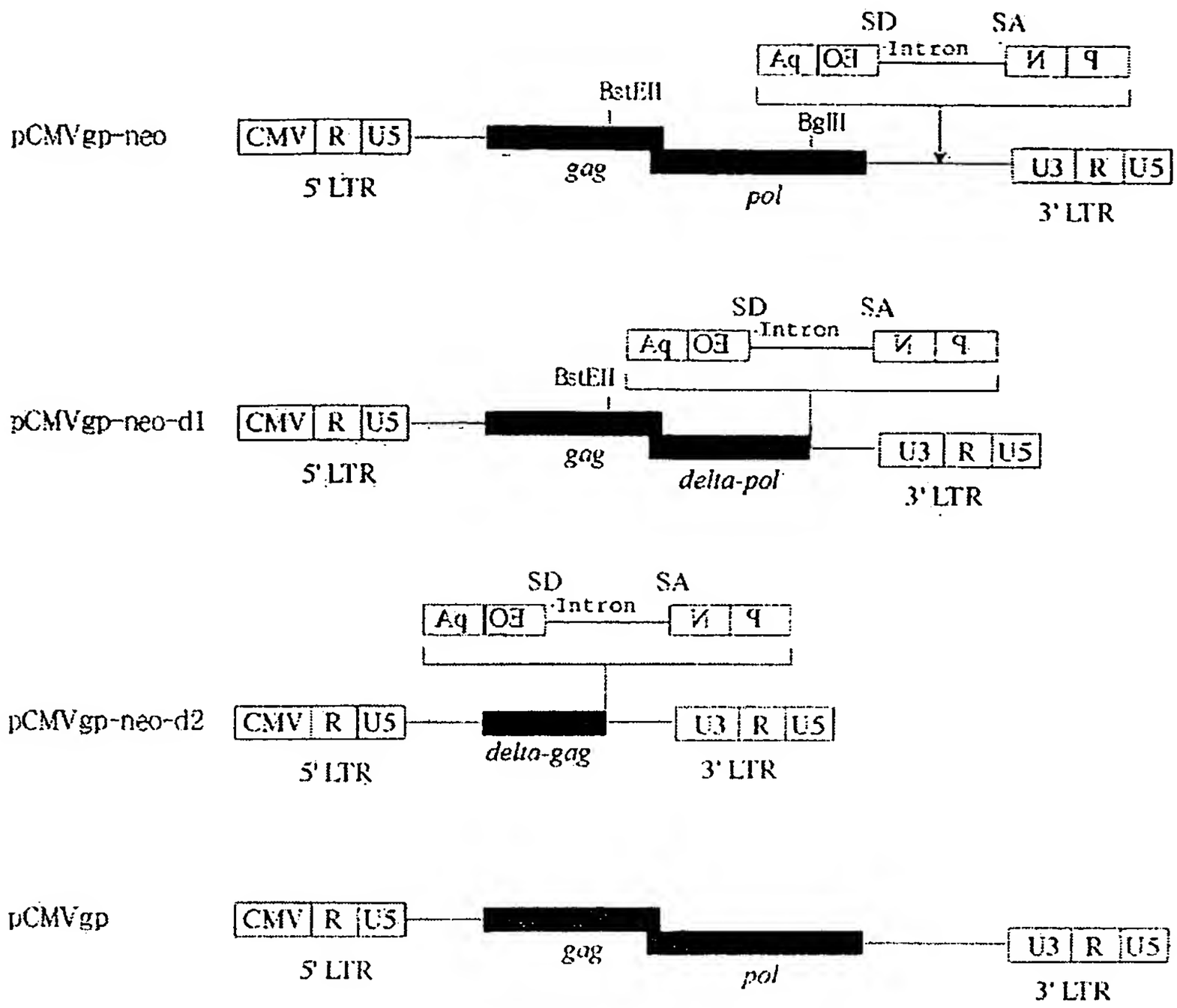


D

Vector	The number of G418 resistant colonies
Mock	0
pU3gp-neo	0
pCMVgp-neo	110
pJM101/L1.3(LINE1)	22

Fig. 4

A



B

Vector	The number of G418 resistant colonies
Mock	0
pCMVgp-neo	264
pCMVgf-neo-d1	0
pCMVgf-neo-d2	0
pCMVgf-neo-d1 + CMVgp	43
pCMVgf-neo-d2 + CMVgp	28

Fig. 5

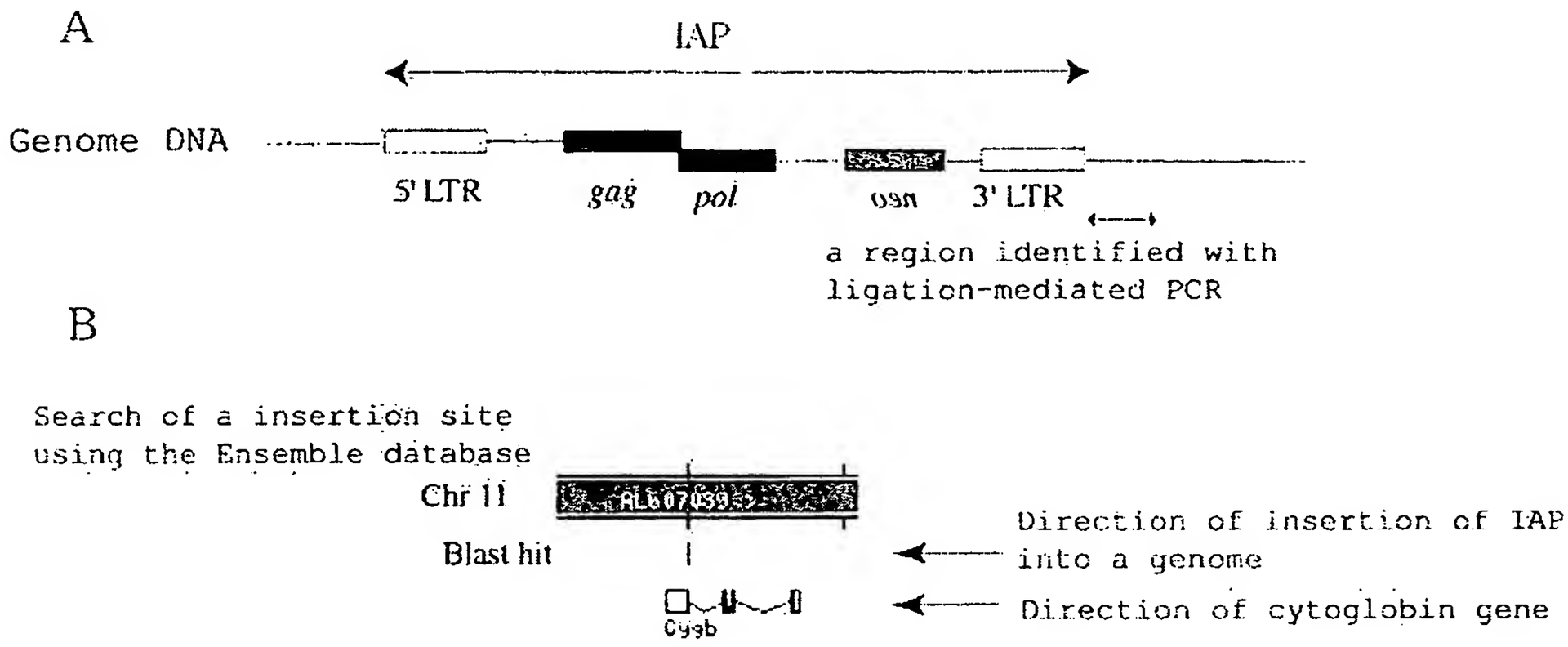
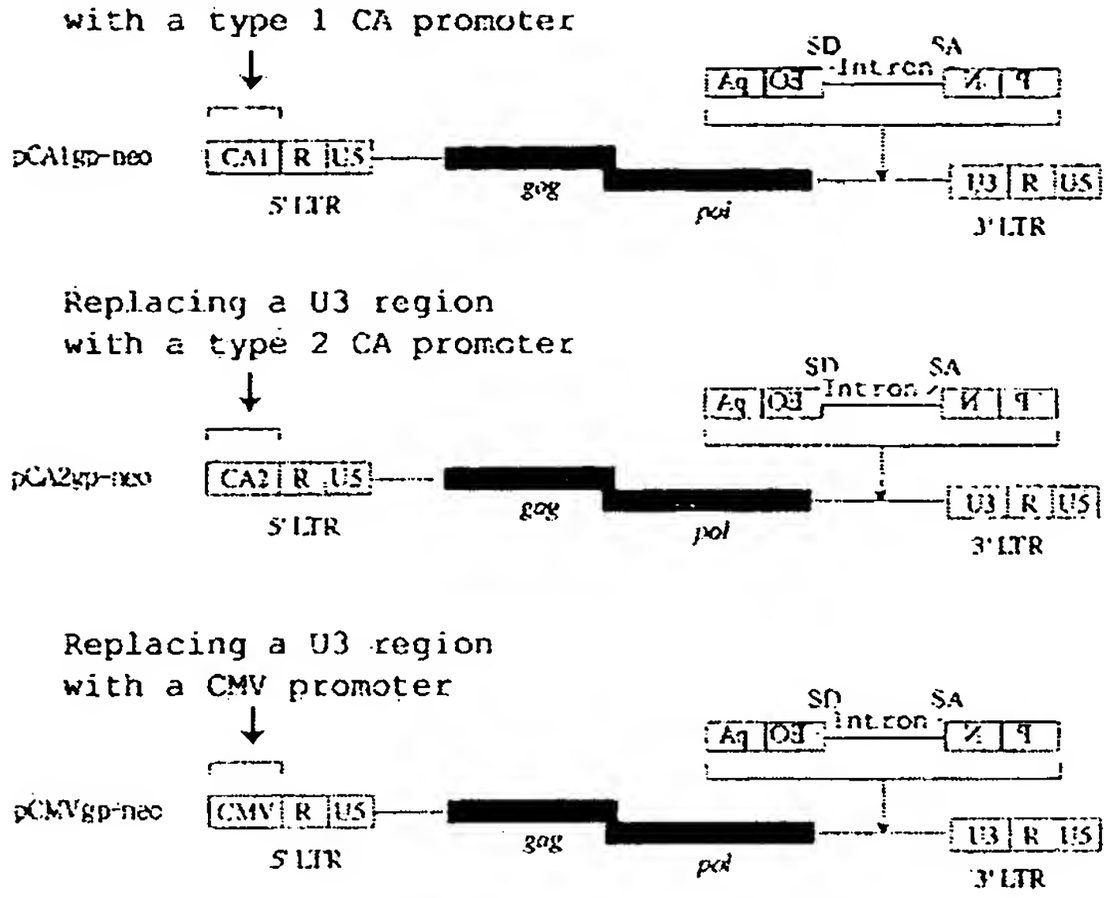
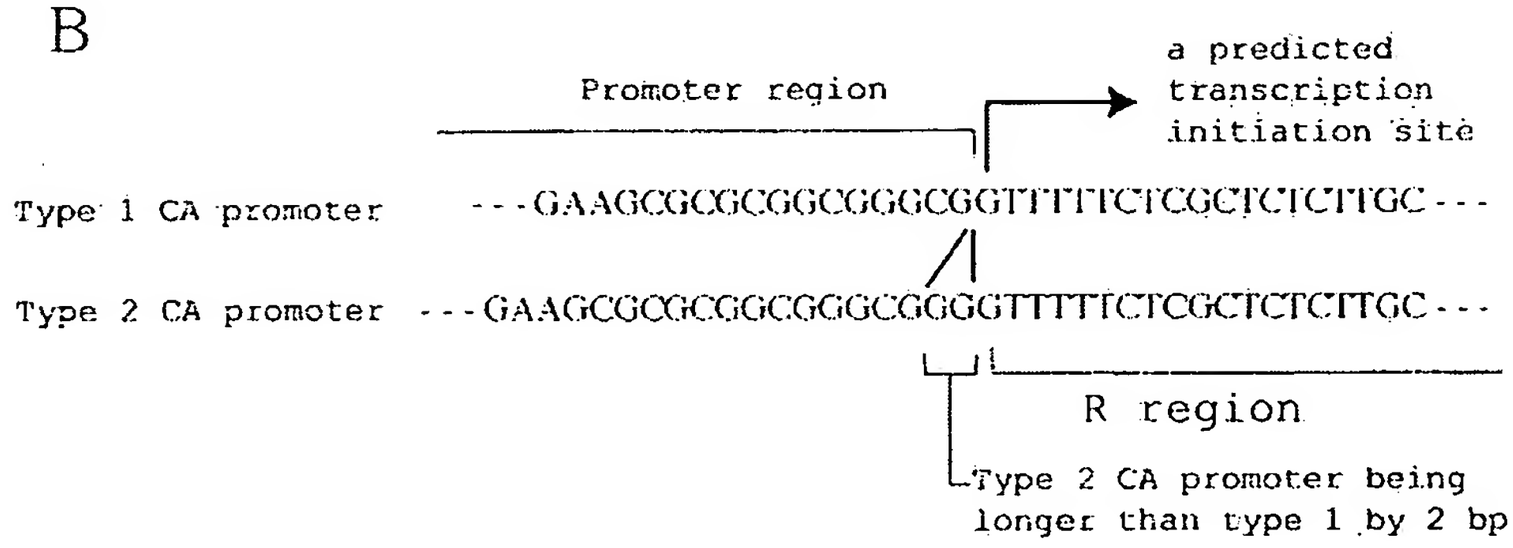

A
IAP
Genome DNA
5' LTR
gag
pol
env
3' LTR
a region identified with
ligation-mediated PCR
B
Search of a insertion site
using the Ensemble database
Chr 11
AL607039
Blast hit
Cygb
Direction of insertion of IAP
into a genome
Direction of cytoglobin gene

Fig. 6

A



B

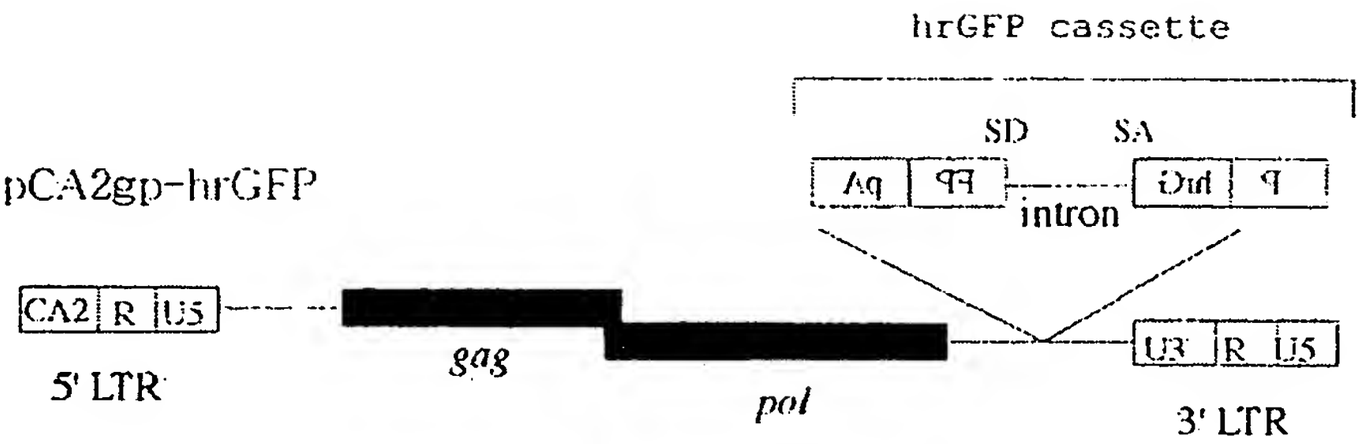


C

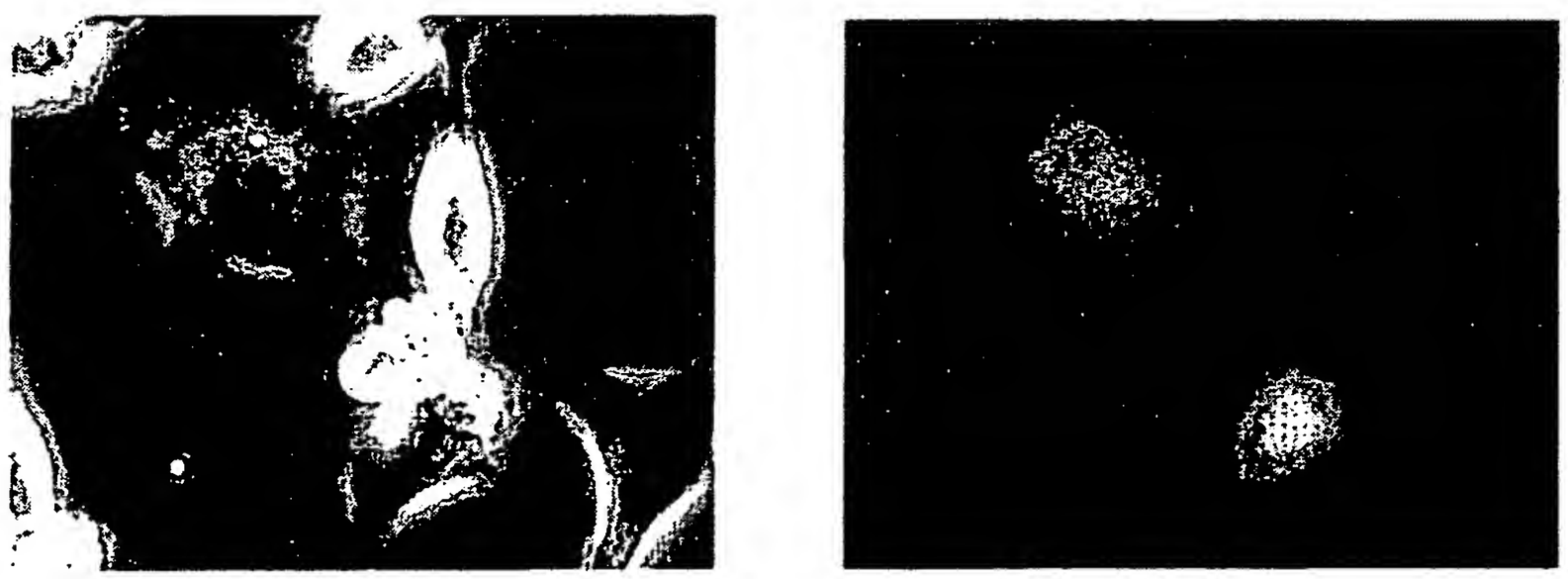
Vector	The number of G418 resistant colonies	
	NIH3T3	HeLa
pCA1gp-neo	65	95
	92	223
pCA2gp-neo	173	230
	124	185
pCMVgp-neo	92	208
	90	177

Fig. 7

A



B



Bright-field Fluorescence-field

Fig. 8

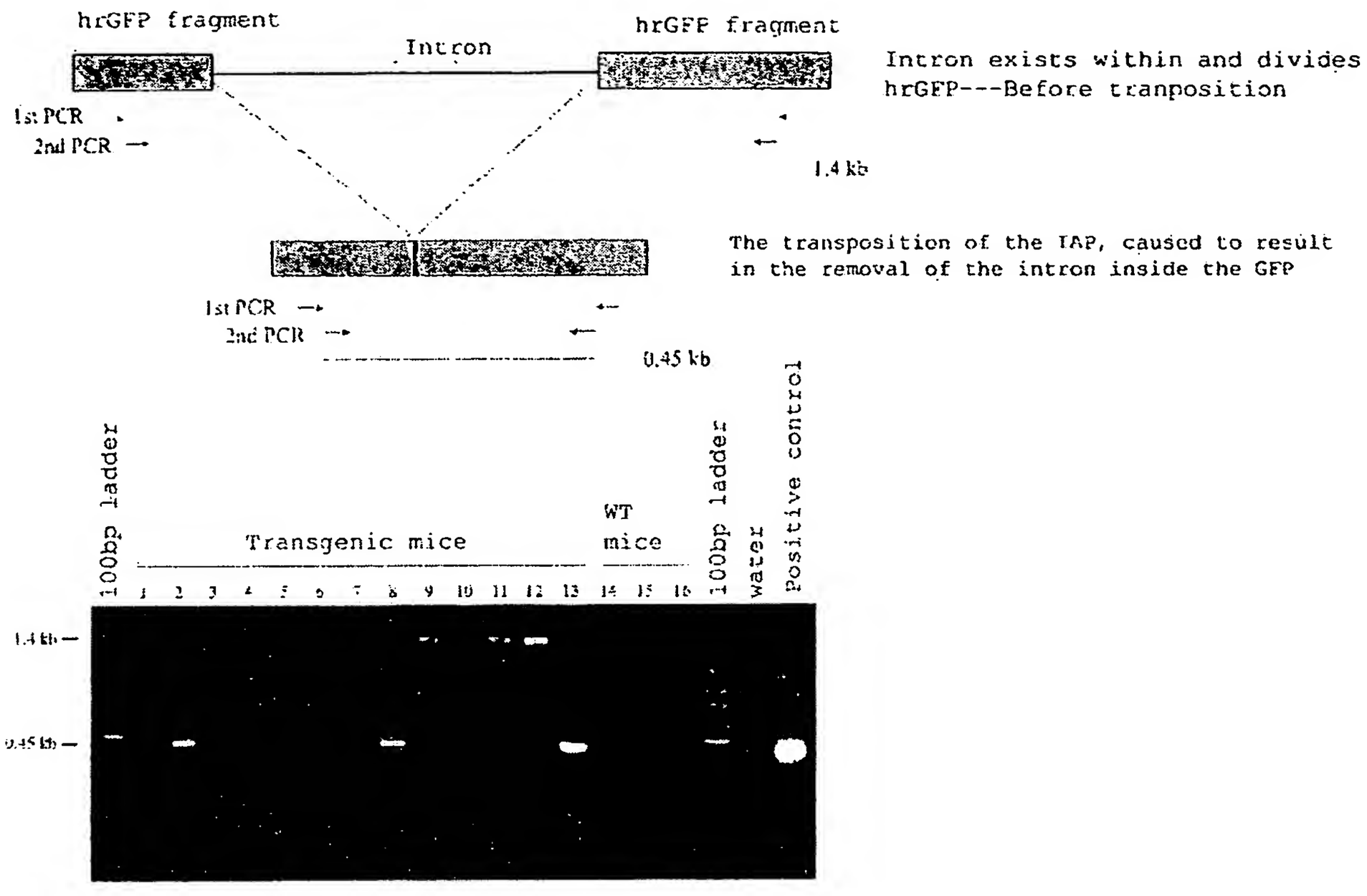
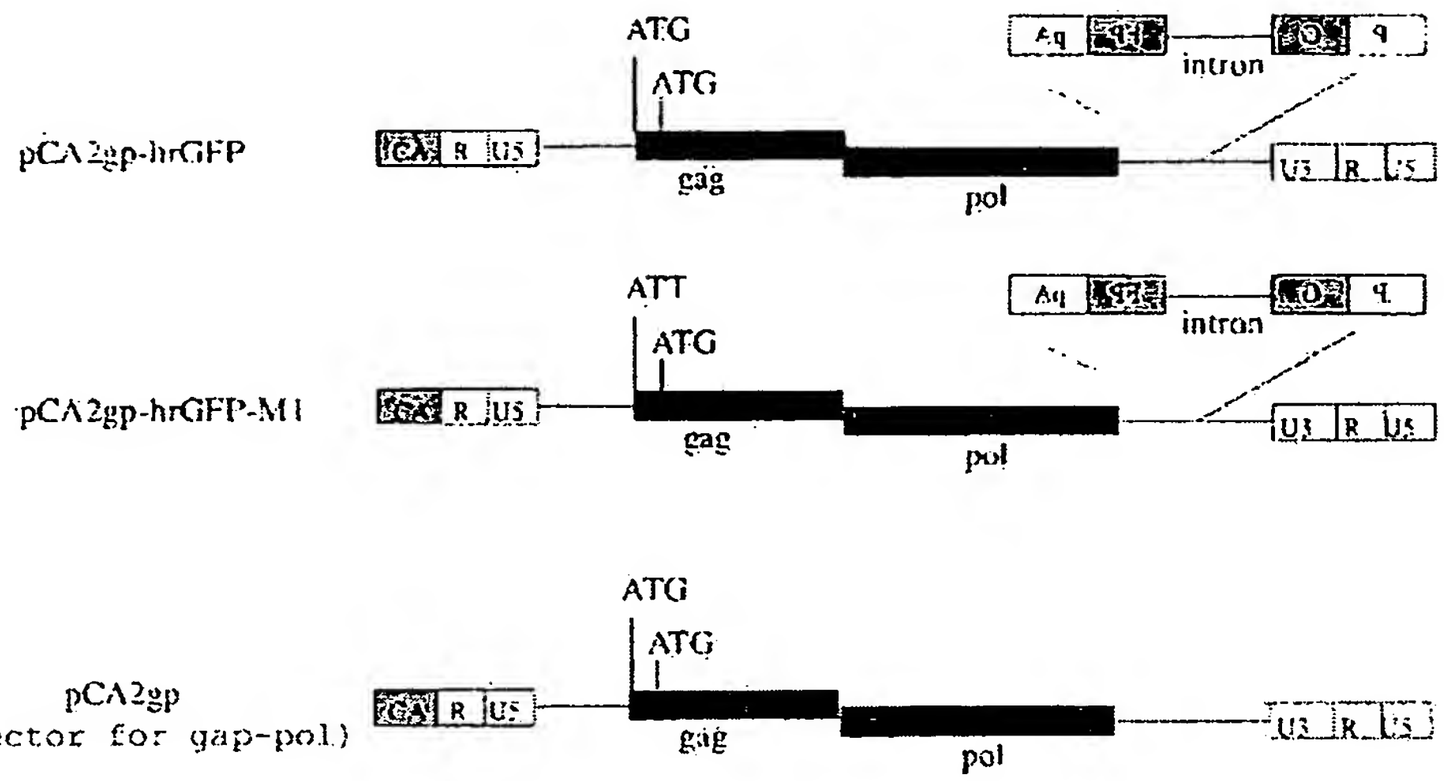

hrGFP fragment
Intron
hrGFP fragment
Intron exists within and divides hrGFP---Before tranposition
1st PCR
2nd PCR
1.4 kb
The transposition of the IAP, caused to result in the removal of the intron inside the GFP
1st PCR
2nd PCR
0.45 kb
100bp ladder
Transgenic mice
WT mice
100bp ladder
water
Positive control
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
1.4 kb
0.45 kb

Fig. 9

A



The first ATG in a gag gene,
which is modified in pCA2gp-hrGFP-M1
into ATT.

The second ATG in a gag gene,
from which translation in pCA2gp-hrGFP-M1
is predicted to start.

WT
(pCA2gp-hrGFP)

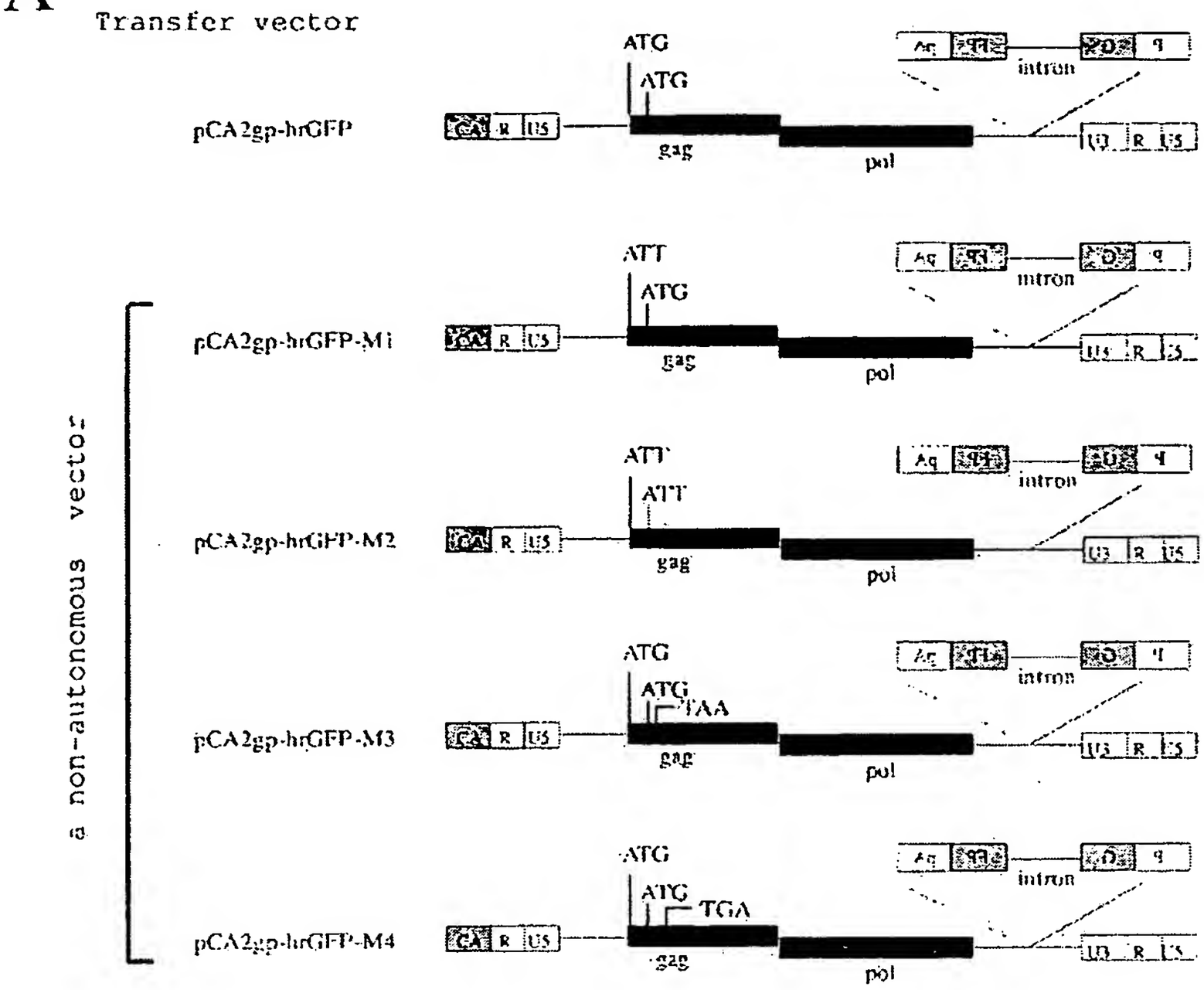
ATG AAT TCA GAA CTT TTC AGC TGG GGA ACG AGA GTA CCA GTG AGT ATG TTT GGC CTT GAA
Met Asn Ser Glu Leu Phe Ser Trp Gly Thr Arg Val Pro Val Ser Met Phe Gly Leu Glu

B

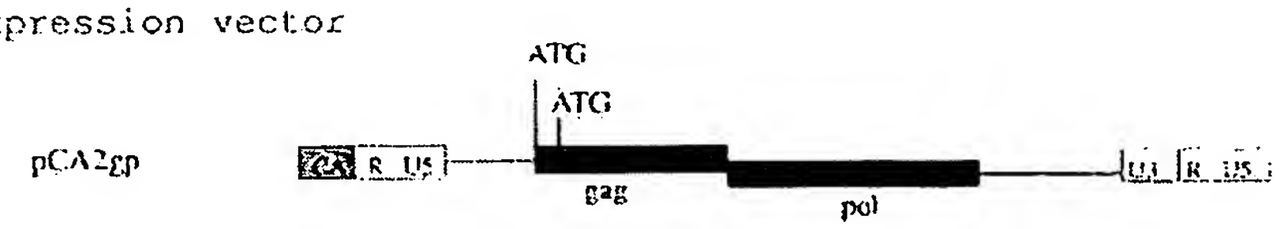
Vector	% GFP-positive
pCA2gp-hrGFP	0.29
pCA2gp-hrGFP-M1	0
pCA2gp-hrGFP-M1 + pCA2gp	0.22

Fig. 10

A



gag-pol expression vector



B

Transfer vector	% GFP-positive	
	+pBluescript	+pCA2gp
pCA2gp-hrGFP	0.29	not done
pCA2gp-hrGFP-M1	0	0.215
pCA2gp-hrGFP-M2	0	0.005
pCA2gp-hrGFP-M3	0	0.04
pCA2gp-hrGFP-M4	0	0.015

Fig. 11